P1 998833

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

April 23, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/368,438*
FILING DATE: *March 27, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/09707*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

03-29-02 A1P80

JC882 U.S. PTO 03/27/02

Docket No. 2026-4363
Express Mail No. EL 853178228 US

JC996 U.S. PTO 60/368430 03/27/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR §1.53(c)(1).

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Hwu	Patrick		11006 Lamplighter Lane, Potomac, MD 20854
Wang	Gang		20220 Darlington Drive, Montgomery Village, MD 20886

TITLE

METHOD FOR TREATING CANCER IN HUMANS

CORRESPONDENCE ADDRESS

☒ Customer Number or Bar Code Label, or
☐ Correspondence address below

27123
PATENT TRADEMARK OFFICE

↑AFFIX CUSTOMER NO. BAR CODE LABEL ABOVE ↑

Name
Address
City State Zip Code
Country Telephone Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification Number of Pages [34]	☐ Small Entity Status is/has been claimed.
☒ Drawings(s) Number of Sheets [6]	☐ Assignment
☒ [28]Claims(s) Number of Sheets [2] *(not required)*	☐ Other:

METHOD OF PAYMENT *(check one)*

☒ A check or money order is enclosed to cover the Provisional filing fees.	PROVISIONAL FILING FEE AMOUNT ($)	☐ $80.00 (small entity)
☐ The Commissioner is hereby authorized to charge the filing fees and credit Deposit Account No. 13-4500, Order No. ____.		☒ $160.00
☒ The Commissioner is hereby authorized to charge any additional fee(s) which may be required, or to credit any overpayment, to Deposit Account No. 13-4500, Order No. 2026-4363.		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No ☒ Yes, the name of the U.S. Government agency and the Government contract number are:

☐ Additional inventors are being named on separately numbered sheets attached hereto

Respectfully submitted,

Signature Dorothy R. Auth Date March 27, 2002

Type or Print Name Dorothy R. Auth Registration No. 36,434

PROVISIONAL APPLICATION FILING ONLY

691292 v1

Docket No. 2026-4363

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Patrick HWU and Gang WANG		
		Group Art Unit:	To be assigned
Serial No.:	To be assigned		
		Examiner:	To be assigned
Filed:	Herewith (March 27, 2002)		

For: METHOD FOR TREATING CANCER IN HUMANS

EXPRESS MAIL CERTIFICATE

Express Mail Label No.: **EL 853178228 US**

Date of Deposit: **March 27, 2002**

I hereby certify that the following attached paper(s) and/or fee

1. Provisional Patent Application containing 37 total pages; 34 pages of specification; 2 pages of claims (1-28); and 1 page of Abstract;
2. Figures 1-6 (6 sheets);
3. Provisional Application Cover Sheet (37 C.F.R. § 1.53(c)(1)), (1 sheet);
4. Application Fee: Check for $160.00; and
5. Return Receipt Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Commissioner for Patents, Box Provisional Patent Application, Washington, DC 20231.

Jesus R. Remedios
(Typed or printed name of person mailing papers(s) and/or fee)

(Signature of person mailing paper(s) and/or fee)

Correspondence Address:
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, NY 10154-0053
(212) 758-4800 Telephone
(212) 751-6849 Facsimile

DOCKET NO.: 2026-4363 PROVISIONAL PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PROVISIONAL PATENT APPLICATION

TITLE: METHOD FOR TREATING CANCER IN HUMANS

INVENTORS: Patrick HWU
Gang WANG

Express Mail Label No. EL853178228US

691546 V1

METHOD FOR TREATING CANCER IN HUMANS

FIELD OF THE INVENTION

[0001] The present invention relates to a method for treating and preventing cancer/malignancies in mammals. More particularly, the present invention is directed to treating cancer by administering an effective amount of IL-21 to a subject, preferably human, in need thereof, such that the effective amount ameliorates, reduces, or eliminates the cancer.

BACKGROUND OF THE INVENTION

[0002] Cytokines are a family of protein mediators of both natural and acquired immunity. They are extracellular proteins that modify the behavior of cells, particularly those cells that are in the immediate area of cytokine synthesis and release. In particular, cytokines are important in regulating hematopoiesis and immune responses. More specifically, cytokines mediate their actions through signal transduction. Accordingly, most cytokines bind to cells and transduce signals through either the class I or class II cytokine receptors. The class I cytokine receptor family includes, but is not limited to, the receptors for interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15, as well as hematopoietic growth factors, leptin and growth hormones, while class II cytokine receptors include the receptors for IL-10 and the interferons (Cosman, D. *Cytokines* 5:95-106, 1993).

[0001] A cytokine most closely related to IL-2 and IL-15 has been identified and is designated IL-21, and its class I receptor is designated IL-21R. Parrish-Novak, et al. suggest that IL-21 plays a role in the proliferation and maturation of natural killer cells from bone marrow, in the proliferation of mature B cells co-stimulated with anti-CD40, and in the proliferation of T cells co-stimulated with anti-CD3 (*Nature* 408:57-63, 2000). Sequencing of the full-length clone, IL-21R, demonstrated that this cDNA contained an open reading frame encoding a 538 amino acid protein having structural motifs common to class I cytokine receptors (Cosman, D. *supra*; Bazan, J.F. *Proc.*

METHOD FOR TREATING CANCER IN HUMANS

FIELD OF THE INVENTION

[0001] The present invention relates to a method for treating and preventing cancer/malignancies in mammals. More particularly, the present invention is directed to treating cancer by administering an effective amount of IL-21 to a subject, preferably human, in need thereof, such that the effective amount ameliorates, reduces, or eliminates the cancer.

BACKGROUND OF THE INVENTION

[0002] Cytokines are a family of protein mediators of both natural and acquired immunity. They are extracellular proteins that modify the behavior of cells, particularly those cells that are in the immediate area of cytokine synthesis and release. In particular, cytokines are important in regulating hematopoiesis and immune responses. More specifically, cytokines mediate their actions through signal transduction. Accordingly, most cytokines bind to cells and transduce signals through either the class I or class II cytokine receptors. The class I cytokine receptor family includes, but is not limited to, the receptors for interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15, as well as hematopoietic growth factors, leptin and growth hormones, while class II cytokine receptors include the receptors for IL-10 and the interferons (Cosman, D. *Cytokines* 5:95-106, 1993).

[0001] A cytokine most closely related to IL-2 and IL-15 has been identified and is designated IL-21, and its class I receptor is designated IL-21R. Parrish-Novak, et al. suggest that IL-21 plays a role in the proliferation and maturation of natural killer cells from bone marrow, in the proliferation of mature B cells co-stimulated with anti-CD40, and in the proliferation of T cells co-stimulated with anti-CD3 (*Nature* 408:57-63, 2000). Sequencing of the full-length clone, IL-21R, demonstrated that this cDNA contained an open reading frame encoding a 538 amino acid protein having structural motifs common to class I cytokine receptors (Cosman, D. *supra*; Bazan, J.F. *Proc.*

Natl. Acad. Sci., USA 87:6934-6938, 1990). Extracellular motifs include a single cytokine recognition module, two pairs of conserved cysteine residues, and a 'WSXWS' motif. The intracellular domain contains strong intracellular signaling motifs, including classical box 1 and box 2 motifs (Murkami, M, et al. *Proc. Natl. Acad. Sci., USA* 88:11349-11353, 1951; Drachman, J.G. and Kaushansky, K. *Proc. Natl. Acad. Sci., USA* 94:2350-2355, 1997; Gurney, A.L., et al. *Proc. Natl. Acad. Sci., USA* 92:5292-5296, 1995), which indicate that the receptor may be a signaling subunit. IL-21R was shown to have the highest amino acid sequence similarity to IL-2R and IL-4Rα. Subsequently, Parrish-Novak, et al. cloned mouse IL-21R from a mouse splenocyte library, and found that it shares overall structure and functional motifs with human IL-21R (Parrish-Novak, et al. *supra*). Further, Parrish-Novak, et al. describe the potent effects of IL-21 on all classes of lymphocytes: B, T, and natural killer cells (Parrish-Novak, et al. *supra*). Additionally, Ozaki, et al. found IL-21R abundantly expressed in lymphoid tissues where expression occurs via the T cell antigen receptor, suggesting that the immune system may play a role (*Proc. Natl. Acad. Sci., USA* 97:11439-11444, 2000).

[0004] Several cytokines known to mediate many of the immune responses involved in antitumor activity have been produced by recombinant DNA methodology and evaluated for their antitumor effects. In clinical trials, the administration of cytokines has resulted in objective tumor responses in patients with various types of neoplasms. More specifically, IL-2, an important cytokine in the generation of antitumor immunity that is structurally related to IL-21, may act locally at the site of tumor antigen stimulation to activate cytotoxic T-cells (CTL) and natural killer cells (NK), cellular immune activity which may mediate systemic tumor cell destruction.

[0005] Intravenous, intralymphatic, or intralesional administration of IL-2 has resulted in clinically significant responses in some cancer patients. However, severe toxicities (e.g., hypotension, pulmonary edema, prerenal azotemia, cardiac arrhythmias and myocardial infarction) limit the dose and efficacy of systemic IL-2 administration. The toxicity of systemically administered cytokines is not surprising since these agents

mediate local cellular interactions and they are normally secreted in limited quantities in a paracrine fashion.

[0006] Despite advances in cancer research, new treatments for cancer are sorely needed. Novel immunotherapy approaches have been devised utilizing cytokines, such as IL-2 and interferon (IFN-α), or cell therapy. However, the toxicity of many of these agents, such as IL-2, is significant. Further, many patients do not respond well to currently available immunotherapeutic and chemotherapeutic agents.

[0007] Therefore, there is a growing need for an effective agent having minimal toxicity for the treatment of cancer.

SUMMARY OF THE INVENTION

[0008] The present invention relates to methods of treating or preventing malignancies/cancer by administering to a patient in need thereof an effective therapeutic amount of an anti-cancer agent to prevent, treat or ameliorate the symptoms of such diseases, disorders, and/or conditions, where the anti-cancer agent is the cytokine, interleukin-21 (IL-21). Such diseases include, for example, melanomas, lymphomas, sarcomas, colon cancer, and the like.

[0009] One embodiment of the invention relates to a method of treating or preventing cancer in a subject, preferably human, comprising: administering an IL-21 protein, polypeptide, or homologue, alone or in combination with a carrier, buffer or saline, in an amount effective to the subject, to treat cancer by ameliorating, reducing, and eliminating cancer.

[0010] A further embodiment relates to a method for treating or preventing cancer in a subject, preferably human, comprising: administering a DNA plasmid alone or in combination with a carrier, buffer or saline, to the subject, wherein the plasmid comprises the full length IL-21 cDNA, and the plasmid and carrier are administered in an effective amount such that uptake of the plasmid occurs, and sufficient expression and secretion of the IL-21 protein results, to treat cancer by

ameliorating, reducing, and eliminating cancer.

[0011] In yet another embodiment, a method for treating or preventing cancer in a subject by administering an effective amount of an IL-21 polypeptide, polynucleotide, vector encoding an IL-21 protein, homologue, variant, or fragment thereof, in combination with an immunotherapeutic and/or chemotherapeutic agent for the treatment and/or prevention of cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the following detailed description when considered in connection with the accompanying drawings wherein:

[0013] FIGURE 1 shows the results of B16 tumor growth inhibition by administering doses of 20 μg mIL-21 compared to 20 μg control.

[0014] FIGURE 2 shows the survival rate of B16 tumor mice treated with 20 μg murine IL-21 (mIL-21) compared to 20 μg control.

[0015] FIGURE 3 shows the results of MC38 tumor growth inhibition by administering 20 μg of mIL-21 compared to 20 μg control.

[0016] FIGURE 4 shows the results of MCA205 tumor growth inhibition by administering 20 μg of mIL-21 compared to 20 μg control.

[0017] FIGURE 5 shows the results of MCA205 tumor growth inhibition by administering doses of 20 μg mIL-21 compared to murine IL-2 (mIL-2; 1 μg) and control (20 μg).

[0018] FIGURE 6 shows the results of MCA205 tumor growth inhibition by administering doses of mIL-21 ranging from 2-20μg compared to mIL-2 (1 μg) and control (20 μg).

DETAILED DESCRIPTION OF THE INVENTION

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0020] "Nucleic acid sequence", as used herein, refers to an IL-21 oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof encoding all or part of Il-21, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded IL-21, and represent the sense or anti-sense strand. By way of non-limiting example, fragments include nucleic acid sequences that are greater than 20-60 nucleotides in length, and preferably include fragments that are at least 70-100 nucleotides, or which are 1000 nucleotides or greater in length.

[0021] As used herein, an IL-21 "polynucleotide" refers to a nucleic acid sequence encoding an IL-21 molecule. For example, the IL-21 polynucleotide may contain the nucleotide sequence of the full-length IL-21 cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence.

[0022] The IL-21 polynucleotide may be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. The IL-21 polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0023] Similarly, "an IL-21 amino acid sequence" as used herein refers to an IL-21 oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules. Amino acid sequence fragments are typically from about 5 to about 30, preferably from about 5 to about 15 amino acids in length and retain the biological activity or function of the IL-21 polypeptide.

[0024] Where amino acid sequence is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, amino acid sequence and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule. Such molecules may also include fusion proteins, chimeric proteins, or other related proteins containing the functional portions of IL-21. In addition, the terms IL-21 polypeptide and IL-21 protein are used interchangeably herein to refer to the encoded product of the IL-21 nucleic acid sequence of the present invention.

[0025] Moreover, as used herein, an IL-21 "polypeptide" refers to a molecule having the translated amino acid sequence generated from the IL-21 polynucleotide as broadly defined. "Secreted" IL-21 protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as an IL-21 protein released into the extracellular space. If the IL-21 secreted protein is released into the extracellular space, the IL-21 secreted protein may undergo extracellular processing to produce a "mature" IL-21 protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

[0026] A "variant" of the IL-21 polypeptide refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "non-conservative" changes, such as, for example, replacement of a glycine with a tryptophan. Minor variations may also include amino acid deletions or insertions, or

both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing functional biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

[0027] An IL-21 polypeptide may have one or more modifications such as but not limited to glycosylation, acetylation, acylation, ADP-ribosylation, methylation, myristoylation, and amidation (Proteins - Structure And Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); Posttranslational Covalent Modification Of Proteins, B. C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

[0028] "Altered" nucleic acid sequences encoding IL-21 polypeptide include nucleic acid sequences containing deletions, insertions and/or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent IL-21 polypeptide. Altered nucleic acid sequences may further include polymorphisms of the polynucleotide encoding the IL-21 polypeptide; such polymorphisms may or may not be readily detectable using a particular oligonucleotide probe. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues, which produce a silent change and result in a functionally equivalent IL-21 protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological activity of IL-21 protein is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

[0029] "Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide ("oligo") linked via an amide bond,

similar to the peptide backbone of amino acid residues. PNAs typically comprise oligos of at least 5 nucleotides linked via amide bonds. PNAs may or may not terminate in positively charged amino acid residues to enhance binding affinities to DNA. Such amino acids include, for example, lysine and arginine, among others. These small molecules stop transcript elongation by binding to their complementary strand of nucleic acid (P.E. Nielsen et al., 1993, *Anticancer Drug Des.*, 8:53-63). PNA may be pegylated to extend their lifespan in the cell where they preferentially bind to complementary single stranded DNA and RNA.

[0030] An IL-21 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-21 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In the case where dose-dependency does exist, it need not be identical to that of the IL-21 polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the IL-21 polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the IL-21 polypeptide).

[0031] One embodiment of the present invention relates to a method of treating or preventing a subject, including, but not limited to dogs, cats, cows, horses, rabbits, monkeys, and most preferably humans, having a cancer, precancer, or immune-related disease, disorder, or condition by administering an effective amount of an IL-21 protein, peptide, or variants thereof, such that the cancer, precancer, or immune-related disease, disorder, or condition is reduced, ameliorated, or eliminated. The IL-21 protein, peptide, or variant thereof, may be in the form of a derivative in which other constituents are attached thereto, such as but not limited to, radioactive labels, biotin, and fluorescein. A targeting agent may also be used to allow for specific targeting to a specific organ, tumor, or cell types. Such targeting agents may be hormones, cytokines, cellular receptors and the like. The IL-21 protein, peptide, or variant thereof, may be administered alone, or in combination with other reagents or

therapeutics.

[0032] Another embodiment of the invention also relates to a method of treating or preventing a cancer, precancer, or immune-related disease, disorder, or condition in a subject having a cancer, precancer, or immune-related disease, by administering a pharmaceutical composition in which an IL-21 protein or peptide is formulated with pharmaceutically acceptable carriers by methods commonly known in the art.

[0033] Yet another embodiment of the present invention relates to a method of treating a subject, preferably mammalian, more preferably human, having a solid tumor or lymphoma, by administering an effective amount of an IL-21 protein, peptide, or variant thereof, a pharmaceutical composition containing an IL-21 protein, peptide or variant thereof, such that the tumor or lymphoma is reduced, ameliorated, or eliminated. Examples of other diseases, disorders, and/or conditions that may be treated include, but are not limited, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, and teratocarcinoma, and particularly, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

[0034] Another embodiment of the present invention relates to a method of treating or preventing a subject, having a cancer, precancer, or immune-related disease, disorder, or condition by administering an IL-21 nucleic acid, polynucleotide, vector comprising a polynucleotide encoding an IL-21 polypeptide, in an amount effective such that the cancer, precancer, or immune-related disease, disorder, or condition is reduced, ameliorated, or eliminated.

[0035] The present invention relates to a method of using a novel anti-cancer agent for treating human cancers, neoplasms and/or malignancies. A plasmid suitable for IL-21 expression has been developed for use as an anti-cancer agent in the treatment of subjects with cancer, such as but not limited to, solid tumors or

lymphomas. One approach relies on the direct administration of recombinant genes into established tumor cells *in vivo*, to genetically modify them, as they grow in situ, to produce and secrete local amounts of IL-21. The secretion of local amounts of IL-21 by tumor cells may cause subsequent tumor reduction or eradication. In the present method, genes may be directly transferred into solid tumor sites where local cells take up and express the gene. In some sites such as skeletal and cardiac muscle, expressible DNA can be injected without using carriers. In other tissues, DNA expression may be facilitated by introducing the DNA complexed with a cationic lipid, such as, for example, in a lipid complex or liposome. The lipid component facilitates the entry of the DNA into those cells provided access to the DNA/lipid complex. Delivery of DNA to patients in a drug-like manner may thereby be facilitated.

[0036] Accordingly, one method of treating or preventing a subject having a cancer may be achieved by inserting a gene encoding IL-21 protein or peptides into high efficiency expression systems, such as *E. coli*, yeast, baculovirus, or vaccinia virus and the like. Techniques using non-viable DNA vectors have the advantage of ease of preparation and safety of administration. The IL-21 nucleic acid sequence is therefore useful as an anti-cancer agent. The DNA sequences encoding the IL-21 proteins or peptides of the present invention may be administered using a gene gun in amounts to elicit a cellular response against a cancer cell. Nanogram quantities are useful for such purposes.

[0037] A further embodiment of the present invention relates to a method of treating or preventing a subject, preferably mammalian, more preferably human, having solid malignant tumors or lymphomas by administering to the subject, an effective amount of an IL-21 plasmid suitable to reduce, ameliorate, and/or eliminate the tumor or lymphoma. The IL-21 plasmid DNA may be directly introduced into the solid tumor cells or nodules of the patient.

[0038] One embodiment relates to a method of treating or preventing a subject having a cancer, precancer, or immune-related disease, disorder, or condition by administrating an IL-21 polypeptide, homologue or variant thereof, polynucleotide,

and/or vector comprising a polynucleotide encoding an IL-21 polypeptide, in combination with other appropriate therapeutic agents in an amount effective to reduce, ameliorate, or eliminate the cancer, precancer, or immune-related disease, disorder, or condition. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

[0039] More specifically, when treating or preventing a solid tumor (either malignant or benign), by administering a plasmid comprising an IL-21 gene, the IL-21 plasmid is directly transferred into solid tumor sites where local cells take up and express the gene. In some sites, such as but not limited to skeletal and cardiac muscle, expressible DNA may be injected without using carriers. In other tissues, such as tumor cells, DNA expression may be facilitated by introducing the DNA and carrier, for example a lipid. The lipid component may facilitate the entry of the DNA into those cells provided access to the DNA/lipid complex. Delivery of the IL-21 DNA to patients in a drug-like manner is thus facilitated.

[0040] In particular, the direct gene transfer approach utilizes a plasmid suitable for IL-2 expression. A preferred plasmid is a circular, double-stranded DNA plasmid that is a simplified eukaryotic expression vector. The gene for IL-21 may be inserted into the plasmid so that IL-21 is expressed when the plasmid is introduced into cells. Other genes may also be included to aid and enhance expression of IL-21. In one embodiment, IL-21 may be inserted into pORF5-mcs (Invivogen) where the multiple cloning sites (mcs) include some of the following restriction sites: Sgr AI, Sal I, Bam HI, Pst I, Nco I, and Nhe I. Accordingly, IL-21 is placed under the transcriptional control of elongation factor 1 α/ eukaryotic initiation factor 4g (EF-1α/eIF4g). Methods, which are well known to those skilled in the art, may be used to construct expression vectors containing sequences encoding the IL-21 polypeptide and

appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in J. Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y. and in F.M. Ausubel et al., 1989, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY.

[0041] A variety of expression vector or host systems may be utilized to contain and express sequences encoding the IL-21 polypeptide. Such expression vector/host systems include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)), or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The host cell employed is not limiting to the present invention.

[0042] "Control elements" or "regulatory sequences" are those non-translated regions of the vector, e.g., enhancers, promoters, 5' and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene; La Jolla, CA) or PSPORT1 plasmid (Life Technologies; Rockville, MD), and the like, may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes), or from plant viruses (e.g., viral promoters or leader sequences), may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferred. If it is necessary to generate a cell line that contains

multiple copies of the sequence encoding IL-21, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

[0043] In bacterial systems, a number of expression vectors may be selected, depending upon the use intended for the expressed IL-21 product. For example, when large quantities of expressed protein are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene; La Jolla, CA), in which the sequence encoding the IL-21 polypeptide may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase, so that a hybrid protein is produced; pIN vectors (see, G. Van Heeke and S.M. Schuster, 1989, *J. Biol. Chem.*, 264:5503-5509); and the like. pGEX vectors (Promega; Madison, WI) may also be used to express foreign polypeptides, as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can be easily purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

[0044] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding the IL-21 polypeptide may be ligated into an adenovirus transcription/ translation complex containing the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the IL-21 polypeptide in infected host cells (J. Logan and T. Shenk, 1984, *Proc. Natl. Acad. Sci., USA* 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

[0045] Specific initiation signals may also be used to achieve more efficient

translation of sequences encoding the IL-21 polypeptide. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the IL-21 polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals, including the ATG initiation codon, may be provided. Furthermore, the initiation codon is preferably in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system that is used, such as those described in the literature (D. Scharf et al., 1994, *Results Probl. Cell Differ.*, 20:125-162).

[0046] In one embodiment, the IL-21 polynucleotide and/or polypeptide, including agonists, antagonists, and fragments thereof, are useful for modulating signaling pathways. In one embodiment of the present invention, an expression vector containing the polynucleotide encoding the IL-21 polypeptide may be administered to an individual to treat or prevent a neoplastic disorder, including, but not limited to, the types of cancers and tumors described above.

[0047] Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy". Thus for example, cells from a subject may be engineered with a polynucleotide, such as DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells can then be introduced into the subject. Further details regarding gene therapy, and specifically on dosage and frequency of cells, are provided in US Patent 5,399,346, which is incorporated herein by reference, *in toto*.

[0048] The genes encoding an IL-21 polypeptide can be turned off by transforming a cell or tissue with an expression vector that expresses high levels of an IL-21 polypeptide-encoding polynucleotide, or a fragment thereof. Such constructs

may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and even longer if appropriate replication elements are designed to be part of the vector system.

[0049] Modifications of gene expression can be obtained by designing antisense molecules or complementary nucleic acid sequences (DNA, RNA, or PNA), to the control, 5', or regulatory regions of the gene encoding an IL-21 polypeptide, (e.g., signal sequence, promoters, enhancers, and introns). Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described (see, for example, J.E. Gee et al., 1994, In: B.E. Huber and B.I. Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, NY). The antisense molecule or complementary sequence may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

[0050] Ribozymes, i.e., enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Suitable examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding IL-21 polypeptide.

[0051] Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the

target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

[0052] Complementary ribonucleic acid molecules and ribozymes according to the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. Such methods include techniques for chemically synthesizing oligonucleotides, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding IL-21. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP. Alternatively, the cDNA constructs that constitutively or inducibly synthesize complementary RNA can be introduced into cell lines, cells, or tissues.

[0053] RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/ or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl, rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytosine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

[0054] Many methods for introducing vectors into cells or tissues are available and are equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods, which are well known in the art.

[0055] A further embodiment of the present invention relates to a method of treating or preventing a cancer, precancer, or immune-related disease, disorder, or condition by administrating a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, diluent, or excipient, for any of the above-described therapeutic uses and effects. Such pharmaceutical compositions may comprise IL-21 nucleic acid, polypeptide, or peptides, activating antibodies to IL-21 receptor, mimetics, agonists, antagonists, or modulators of IL-21 polypeptide or polynucleotide. In a further instance, it may be useful to administer pharmaceutical compositions comprising neutralizing or inhibitory antibodies to IL-21 receptor for the treatment of autoimmune diseases or instances where inhibiting IL-21 receptor and ligand interactions have beneficial effects, as exemplified by blocking CD40-CD40L interactions (Diehl, et al. *J. Mol. Med.* 78:363-371, 2000; Datta, S.K. and S.L. Kalled *Arthritis & Rheumatism* 40:1735-1745, 1997). The compositions may be administered alone, or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, hormones, or biological response modifiers.

[0056] In a further embodiment, the proteins, activating antibodies, agonists or modulators, complementary sequences, or vectors of the present invention can be administered alone, or in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

[0057] In particular, the combination approach relates to tumor cells that are collected, propagated *in vitro*, modified and selected and then reinjected *in vivo*. More

specifically, this embodiment relates to passive immunotherapy with genetically modified immune cells (commonly referred to as adoptive immunotherapy) capable of recognizing human tumor antigens effective in mediating the regression of cancer in selected patients with metastatic melanoma. *In vitro* techniques have been developed in which human lymphocytes are sensitized *in vitro* to tumor antigen immunodominant peptides presented on antigen presenting cells. Administration of IL-21 polypeptide or polynucleotide may be used in conjunction to increase the effects of adoptive immunotherapy for the treatment and/or prevention of cancer in a subject.

[0058] T cells from immunized mammals that have specific reactivity against cancer, precancer, or an immune-related disease, disorder, or condition, may also be used *in vivo* for the treatment of individuals afflicted with cancer by administering from about 10^7 to 10^{11} T cells to a mammal intravenously, intraperitoneally, intramuscularly, or subcutaneously in addition to IL-21 polypeptide or polynucleotide. Preferred routes of administration are intravenously or intraperitoneally.

[0059] For example, incorporation of the gene for IL-2 can increase the immunogenicity of tumor antigens and even mediate the regression of established lung metastases bearing these antigens and even mediate the regression of established lung metastases bearing these antigens. Active immunotherapy followed by the exogenous administration of co-immunostimulatory cytokines such as IL-2, IL-6, IL-10, and preferably IL-21 may also be used to improve immune responses.

[0060] Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with IL-21 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect the mammal from infections, such as bacterial, fungal, protozoan and viral infections, or infections caused by HIV-1 or HIV-2, cancer, precancer, and other immune-related diseases, disorders, or conditions. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering IL-21 polypeptide via a vector directing

expression of IL-21 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect the mammal from the diseases, disorders, or conditions described above.

[0061] A further aspect of the invention relates to an immunological or vaccine formulation or composition which, when introduced into a mammalian host, induces an immunological response in the mammal to an IL-21 polypeptide, where the composition comprises an IL-21 polypeptide or IL-21 gene. The formulation may further comprise a suitable carrier. Since the IL-21 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intravenous, intramuscular, intradermal, etc., injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in-water systems and other systems known in the art. Furthermore, IL-21 may also be used as an adjuvant either alone or in combination with other reagents to enhance the immunization or vaccination against cancer, precancer, and/or immune-related diseases, disorders, or conditions. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Administration

[0062] The present invention therefore relates to methods of preventing or inhibiting cancer, precancer, preferably tumors or lymphomas, and immune-related diseases, disorders, or conditions in mammals, by administering IL-21 protein or peptides (or nucleic acid sequences encoding them) to the mammal via routes of

administration that include, but are not limited to intravenous, subcutaneous, intratumor, intramuscular, intradermal, intraperitoneal, intrathecal, intraplerural, intrauterine, rectal, vaginal, topical, and the like.

[0063] Administration may also be by transmucosal or transdermal means. For transmucosal or transdermal administration penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be by nasal sprays, for example, or suppositories. For oral administration, the IL-21 protein, peptides, or variants thereof are formulated into conventional oral administration forms such as capsules and tablets.

[0064] In addition to administering IL-21 molecules alone, pharmaceutical compositions comprising a therapeutically effective amount of one or more IL-21 molecules in a mixture with a pharmaceutically acceptable carrier may be used. This composition can be administered either parenterally, intravenously or subcutaneously. When administered, the therapeutic composition for use in this invention is preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

[0065] The pharmaceutical compositions for use in the present invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, vaginal, or rectal means.

[0066] In addition to the active ingredients (i.e., an IL-21 nucleic acid and/or polypeptide, and/or functional fragments thereof, US/2001/0023070 A1 to Ebner et al. which is hereby incorporated by reference, *in toto*), the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers or excipients comprising

auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration are provided in the latest edition of Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA).

[0067] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

[0068] Pharmaceutical preparations for oral use can be obtained by the combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropyl-methylcellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth, and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a physiologically acceptable salt thereof, such as sodium alginate.

[0069] Dragee cores may be used in conjunction with physiologically suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification, or to characterize the quantity of active compound, i.e., dosage.

[0070] Pharmaceutical preparations, which can be used orally, include push-fit capsules made of gelatin, as well as soft, scaled capsules made of gelatin and a

coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

[0071] Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. In addition, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyloleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0072] For topical or nasal administration, penetrants or permeation agents that are appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0073] The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

[0074] The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, and the like. Salts tend to be more soluble in aqueous solvents, or other protonic solvents, than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain

any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, combined with a buffer prior to use. After the pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of IL-21 product, such labeling would include amount, frequency, and method of administration.

[0075] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose or amount is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., using neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used and extrapolated to determine useful doses and routes for administration in humans.

[0076] A therapeutically effective dose refers to that amount of active ingredient, for example, IL-21 polypeptide, or fragments thereof, activating antibodies to IL-21 receptor, agonists, or modulators of IL-21 polypeptide, which ameliorates, reduces, or eliminates the cancer, precancer, or immune-related disease, disorder, or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio, LD_{50}/ED_{50}. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in determining a range of dosages for human use. Preferred dosage contained in a pharmaceutical composition is within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity

of the patient, and the route of administration.

[0077] The practitioner, who will consider the factors related to the individual requiring treatment, will determine the exact dosage. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors, which may be taken into account, include the severity of the individual's disease state, general health of the patient, age, weight, and gender of the patient, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/ response to therapy. As a general guide, long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks, depending on half-life and clearance rate of the particular formulation. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

<u>Dosages</u>

[0078] Normal dosage amounts may vary from 0.1 to 100,000 micrograms (μg), up to a total dose of about 1 gram (g), depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and is generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, and the like.

[0079] Generally, it is desirable to provide the recipient with a dosage of IL-21 protein or peptide of at least about 1 pg/ kg body weight, preferably at least 1 ng/ kg body weight, more preferably at least about 1 g/ kg body weight or greater of the recipient. A range of from about 1 μg/ kg body weight to about 100 mg/ kg body weight is preferred, and a range from 10 μg/kg body weight to 10 mg/kg body weight is more preferred, although a lower or higher dose may be administered. The desired dose is effective to prevent or inhibit cancer, precancer, and immune-related diseases, disorders, or conditions in the recipient, preferably human.

[0080] The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician considering various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, a daily regimen may be in the range of about 1 mg to about 2.5 mg of IL-21 plasmid DNA per kilogram of body weight. Dosages would be adjusted relative to the activity of, for example, IL-21 and it would not be unreasonable to note that dosage regimens may include doses as low as 1 microgram and as high as 5 milligram per kilogram of body weight per day. In addition, there may exist specific circumstances where dosages of IL-21 would be adjusted higher or lower than this range. For example, when IL-21 is used as an adjuvant, the dose may be much lower, such as 1 microgram per kilogram body weight or per injection site. These include co-administration with other anti-cancer agents and/or co-administration with chemotherapeutic drugs and/or radiation. As indicated above, the therapeutic method and compositions may also include co-administration with other human factors. A non-exclusive list of other appropriate hematopoietins, CSFs, cytokines, lymphokines, hematopoietic growth factors and interleukins for simultaneous or serial co-administration with the polypeptides of the present invention includes GM-CSF, CSF-1, G-CSF, Meg-CSF (more recently referred to as c-mpl ligand), M-CSF, erythropoietin (EPO), IL-1, IL-4, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, LIF, flt3 ligand, B-cell growth factor, B-cell differentiation factor and eosinophil differentiation factor, stem cell factor (SCF) also known as steel factor or c-kit ligand, or combinations thereof. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by periodic assessment of the cancer profile, e.g., blood cell count and the like.

[0081] All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein,

including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

[0082] Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

[0083] The invention will now be illustrated by the following non-limiting examples.

EXAMPLE 1

CLONING OF HUMAN AND MURINE IL-21

[0084] Human PBMC and murine spleen cells (C57BL/6) were activated by 5 ng/ml of PMA and 250 μg/ml Ionomycin for 24 hr. Total RNA was extracted and isolated by TRIZOL method (Life Technologies/Invitrogen; Carlsbad, CA). RT-PCR was performed to amplify the first strand of cDNA by random primers according to manufacturer's instruction (ThermoScript RT-PCR System; Life Technologies/ Invitrogen). The full length cDNA fragments (including the original signal peptide) was PCR amplified using a pair of specific primers for either human or murine IL-21.

[0085] The human IL-21 primers used are as follows:

Human Forward: 5'-cca-ccg-gcg-gta-ctt-atg-aga-tcc-agt-cct-ggc-3' SEQ ID NO:1
Human Reverse: 5'-gct-agc-tca-gga-act-ttc-act-tcc-gtg-3' SEQ ID NO:2

[0086] The murine IL-21 primers used are as follows:

Murine Forward: 5'-cca-ccg-gcg-ggt-ggc-atg-gag-agg-acc-ctt-gtc-3' SEQ ID NO:3
Murine Reverse: 5'-gct-agc-cta-gga-gag-atg-ctg-atg-aat-3' SEQ ID NO:4

[0087] The PCR amplified DNA fragments were cloned into TA Cloning

vectors. One clone from human IL-21 and three clones from mouse IL-21 were obtained. These clones were verified by DNA sequencing. The one human IL-21 clone had two mutations in its sequence. Two of the murine clones had the correct sequence, and the third clone had 1 mutation in its sequence. The murine IL-21 cDNA fragments with the correct sequences were digested with Sgr AI and Nhe I, and cloned into the pORF5-mcs vector (InvivoGen; San Diego, CA). A large preparation of plasmid DNA was isolated using the Endofree™ Plasmid Mega purification kit by Qiagen, Inc. (Valencia, CA).

EXAMPLE 2

ANTITUMOR ACTIVITY OF mIL-21 ON B16 TUMOR MICE

[0088] On day 0, C57BL/6 mice (5 mice per group) were subcutaneously injected with B16 mouse melanoma cells (5 x 10^5/mouse). On day 5, tumor-bearing mice were intravenously injected with different doses of mIL-21 plasmid DNA ranging from 5 to 40μg in 2 ml of saline. Control mice (5 mice/control) were injected with 20 μg of control plasmid DNA (vector alone) in 2 ml of saline. Seven days later, the mIL-21 and control injections were repeated once more. Tumor growth was monitored thereafter by measuring tumor sizes twice a week. Results showing inhibition of B16 tumor growth by administering doses of 20 μg mIL-21 are shown in Figure 1.

EXAMPLE 3

ANTITUMOR EFFECT OF mIL-21 ON B16 TUMOR MICE SURVIVAL

[0089] On day 0, C57BL/6 mice (5 mice per group) were subcutaneously injected with B16 mouse melanoma cells (2 x 10^5/mouse). On day 5, tumor-bearing mice were intravenously injected with either 20 μg of control plasmid DNA (vector alone) or 20 μg of mIL-21 plasmid DNA in 2 ml of saline. Eight days later, the mIL-21 and control injections were repeated once more. Tumor growth was monitored thereafter by measuring tumor sizes twice a week. The survival rate was also

recorded. On day 28, all 5 control mice had tumors (2 had died); and 4 of the 5 mice mIL-21 treated mice were tumor free, only 1 mouse had a tumor (see Figure 2).

EXAMPLE 4

ANTITUMOR ACTIVITY OF mIL-21 ON MC38 AND MCA205 TUMOR MICE

[0090] C57BL/6 mice (5 mice / group) were subcutaneously injected with MC38 and MCA205 tumor cells on day 0. Specifically, MC38 mouse sarcoma cells ($3x10^3$/ mouse) were injected on the left side and MCA205 mouse colon cancer cells (5 x 105/ mouse) on the right. On day 3, tumor-bearing mice were intravenously injected with different doses of mIL-21 plasmid DNA ranging from 0 to 40 μg in 1.5 ml of saline. Vector alone in saline and saline alone were used as controls. Seven days later, the mIL-21 injection was repeated once more. Tumor growth was monitored thereafter by measuring tumor sizes twice a week. Results from this ongoing repeat experiment are shown in Figures 3 and 4.

EXAMPLE 5

ANTITUMOR ACTIVITY OF mIL-21 ON MCA205 TUMOR MICE

[0091] On day 0, C57BL/6 mice (5 mice per group) were subcutaneously injected with MCA205 mouse colon cancer cells (2 x 10^5/mouse). On day 5, tumor-bearing mice were intravenously injected with either 20 μg of control plasmid DNA (vector alone), 5 to 20 μg of mIL-21 plasmid DNA, or 1 μg of mIL-2 plasmid DNA in 1.5 ml of saline. Six days later, the mIL-21 and control injections were repeated once more in 2 ml of saline. Tumor growth was monitored thereafter by measuring tumor sizes twice a week. Results from this repeat experiment are shown in Figures 5 and 6.

EXAMPLE 6

EFFECT OF mIL-21 ON MOUSE COMPLETE BLOOD COUNT AND CYTOKINE SECRETION

[0092] On day 0, C57BL/6 mice (3 mice per group) were intravenously

injected with either saline alone, 20 μg of control plasmid DNA (vector alone), or 20 μg of mIL-21 plasmid DNA in 2 ml saline. On days 1, 4, and 7, mouse blood was collected from the tail vein and subjected to complete blood count (CBC) analysis (see Table1). Serum was also collected for multiple cytokine immunoassays with LINCOplex™ mouse cytokine panels (LINCO Research, Inc.; St. Louis, MO) and Luminex system (Luminex Corp.; Austin, TX) (see Table 2). Mouse spleen and liver tissues were harvest for Haematoxylin and Eosin (H&E;) staining and analysis. Splenocytes were analyzed by fluorescence activated cell sorter (FACS) for various cell surface markers (see Table 3).

[0093] The experiment was repeated using a modified protocol where on days 1, 4, 8, and 14, mouse blood was collected from the tail vein and subjected to CBC analysis (see Table 1). Mouse serum was subsequently collected for multiple cytokine immunoassays with LINCOplex™ mouse cytokine panels and the Luminex system (see Table 2).

EXAMPLE 7

COMPLETE BLOOD COUNTING

[0094] The complete blood counting (CBC) analysis was performed by the Department of Laboratory Medicine, Clinical Center at the National Institutes of Health (NIH). An Abbott's Cell-Dyn® 3500 automated hematology analyzer, which employs dual technologies to provide the basis for a five-part white blood cell count differential, was used . Twenty microliters of fresh mouse whole blood was diluted to a final volume of 200 μl of cell suspension by adding 180 μl of phosphate buffered saline (PBS) for sample preparation. The cell suspension was then transferred to the Microtainer® tubes (Becton Dickinson) with EDTA, and sent to the clinical lab for analysis.

EXAMPLE 8

CYTOKINE SECRETION ASSAYS AND CYTOKINE IMMUNOASSAYS

[0095] The cytokine secretion experiment was performed by injecting 20 μg of either control or mIL-21 plasmid DNA intravenously into normal C57BL/NCR mice. At the different time points after injection, mouse blood samples were collected by tail vein bleed. Serum samples were subsequently collected and subjected to mouse cytokine analysis by the Mouse Cytokine Multiplex Kit (Linco Research, Inc.). The analysis was performed by Linco Research, Inc. using the LincoPlex™ and the Luminex 100™ System. Sixty microliters of mouse serum from each sample was sent to Linco Research, Inc. for analysis using a multiple panel of mouse cytokines, including but not limited to, Il-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IFN-γ, TNF-α, and GM-CSF. Assay conditions included overnight assay for serum/plasma using 25 μl sample volume. The cytokine antibody pairs in the multiplex do not cross react to the other analytes in the panel. Also, the antibody pairs have less than 1% cross reactivity with IL-1α, IL-3, IL-7, INF-β, IFN-β, IFN-α, MIP-1α, MIP-1β, and MCP-1. Sensitivity for mIL-1β, mIL-2, mIL-4, mIL-5, mIL-6, mIL-10, and mIFN-γ is 3.2 pg/ml and mIL-12(p70), mTNF, and mGM-CSF is 16 pg/ml in 10% fetal bovine serum.

[0096] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the

skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

TABLE 1:

Effect of mIL-21 on mouse blood CBC counts (average of 3 mice/group)

Exp 1, day 4	Saline	Ctr	mIL-21	No Tx
WBC count (K/ul)	1.447	1.633	1.459	1.500
Lymphocytes (%)	89.1	90.9	83.4	89.1
Monocytes (%)	4.9	3.8	9.6	3.1
Lympho absolute (K/ul)	1.290	1.486	1.244	1.335
Mono absolute (K/ul)	0.069	0.062	0.133	0.048
Exp 1, day 7	**Saline**	**Ctr**	**mIL-21**	**No Tx**
WBC count (K/ul)	0.266	0.180	0.219	0.123
Lymphocytes (%)	80.8	78.0	39.2	78.7
Monocytes (%)	6.7	10.9	32.1	7.1
Lympho absolute (K/ul)	0.215	0.141	0.079	0.096
Mono absolute (K/ul)	0.018	0.019	0.065	0.009
Exp 2, day 1	**Saline**	**Ctr**	**mIL-21**	**No Tx**
WBC count (K/ul)	0.816	0.892	0.461	0.737
Lymphocytes (%)	77.1	80.1	71.4	87.4
Monocytes (%)	13.7	4.4	5.5	3.9
Lympho absolute (K/ul)	0.572	0.717	0.348	0.647
Mono absolute (K/ul)	0.086	0.035	0.026	0.003
Exp 2, day 4	**Saline**	**Ctr**	**mIL-21**	**No Tx**
WBC count (K/ul)	0.909	0.812	1.215	0.612
Lymphocytes (%)	79.2	85.7	81.3	90.6
Monocytes (%)	6.8	7.1	11.6	3.3
Lympho absolute (K/ul)	0.726	0.688	0.983	0.553
Mono absolute (K/ul)	0.060	0.056	0.145	0.020

Express Mail Label No. EL853178228US

691546 V1

TABLE 2:

Effect of mIL-21 on mouse serum cytokine secretion (LINCOplex™ immunoassay)

No	Treatment	Day	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IFN-γ	GM-CSF	TNF-α
1	saline	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
2	saline	1	<3	<3	<3	<3	<3	<3	<16	5	40	<16
3	saline	1	<3	<3	<3	19	<3	<3	<16	7	<16	<16
4	control	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
5	control	1	<3	<3	<3	7	<3	<3	<16	33	236	<16
6	control	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
7	mIL-21	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
8	mIL-21	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
9	mIL-21	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
10	no Tx	1	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
11	no Tx	1	<3	<3	<3	6	4	18	<16	<3	<16	<16

No	Treatment	Day	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IFN-γ	GM-CSF	TNF-α
1	saline	4	<3	<3	<3	7	<3	<3	<16	4	<16	<16
2	saline	4	<3	<3	<3	<3	<3	<3	<16	12	60	<16
3	saline	4	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
4	control	4	<3	<3	<3	<3	8	179	205	114	466	672
5	control	4	<3	<3	<3	7	<3	<3	<16	58	361	<16
6	control	4	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
7	mIL-21	4	<3	<3	<3	22	<3	<3	<16	<3	<16	<16
8	mIL-21	4	<3	<3	<3	18	<3	<3	<16	<3	<16	<16
9	mIL-21	4	<3	<3	<3	27	10	16	<16	<3	<16	<16
10	no Tx	4	<3	<3	<3	<3	10	<3	<16	<3	<16	<16
11	no Tx	4	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16

No	Treatment	Day	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IFN-γ	GM-CSF	TNF-α
1	saline	7	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
2	saline	7	<3	<3	<3	<3	5	<3	<16	<3	<16	<16
3	saline	7	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16
4	control	7	<3	<3	<3	<3	<3	<3	<16	26	96	39
5	control	7	<3	<3	<3	<3	<3	<3	<16	10	58	<16
6	control	7	<3	<3	<3	<3	4	<3	<16	4	<16	<16
7	mIL-21	7	<3	<3	<3	13	<3	<3	<16	<3	<16	47
8	mIL-21	7	<3	<3	<3	14	<3	<3	<16	<3	<16	<16
9	mIL-21	7	<3	<3	<3	<3	31	29	<16	<3	<16	<16
10	no Tx	7	<3	<3	<3	<3	<3	<3	<16	4	<16	<16
11	no Tx	7	<3	<3	<3	<3	<3	<3	<16	<3	<16	<16

TABLE 3

Effect of mIL-21 on cell surface markers of mouse splenocytes

	Saline-1	Saline-2	Saline-3	Saline-Ave	Ctr-1	Ctr-2	Ctr-3	Ctr-Ave	mIL-21-1	mIL-21-2	mIL-21-3	mIL-21-Ave	No tx-1	No Tx-2	No Tx-Ave
CD4	15.01	10.84	9.33	11.73	6.44	13.10	12.88	10.81	12.66	11.19	13.81	12.55	12.43	10.79	11.61
CD4/CD25	3.18	1.80	1.16	2.05	0.95	1.87	1.83	1.55	2.16	1.98	2.56	2.23	2.01	1.71	1.86
CD8	14.43	11.48	10.39	12.10	9.10	14.79	10.84	11.58	15.29	18.17	18.52	17.33	11.71	11.25	11.48
CD25	3.54	2.19	1.58	2.44	1.33	2.31	2.33	1.99	2.81	2.68	3.29	2.93	2.54	2.34	2.44
CD45	93.43	79.77	84.03	85.74	87.46	87.51	85.71	86.89	82.49	80.47	86.19	83.05	90.87	93.45	92.16
NK1.1	4.18	3.57	4.15	3.97	4.66	3.71	3.39	3.92	2.84	2.81	2.99	2.88	4.89	5.65	5.27
B220	53.18	54.02	58.85	55.35	58.23	53.63	55.75	55.87	40.39	29.90	31.47	33.92	62.14	62.99	62.57
CD11c	3.90	3.21	4.20	3.77	4.43	3.37	3.49	3.76	4.13	5.28	4.56	4.66	3.07	3.29	3.18
CD14	1.11	1.24	1.00	1.12	0.83	0.75	1.19	0.92	0.86	1.05	1.07	0.99	0.92	1.21	1.07
CD11b	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01
CD62L	0.01	0.03	0.04	0.03	0.05	0.04	0.00	0.03	0.00	0.01	0.00	0.00	0.01	0.03	0.02

WHAT IS CLAIMED IS

1. A method of treating a cancer in a mammal, comprising administering an IL-21 polypeptide, homologue, or fragment thereof, in an amount effective to treat the cancer.

2. A method of treating a cancer in a mammal, comprising administering an IL-21 polynucleotide or fragment thereof, in an amount effective to treat the cancer.

3. A method of treating a cancer in a mammal, comprising administering an expression vector containing an IL-21 polynucleotide, in an amount effective to treat the cancer.

4. The method according to claim 3, wherein the expression vector is pORF.

5. The method according to claim 1, 2, or 3, wherein the cancer is a melanoma.

6. The method according to claim 1, 2, or 3, wherein the cancer is a sarcoma.

7. The method according to claim 1, 2, or 3, wherein the cancer is a colon cancer.

8. A method of treating an immune-related disease in a mammal, comprising administering an IL-21 polypeptide, homologue, or fragment thereof, in an amount effective to treat the immune-related disease.

9. A method of treating an immune-related disease in a mammal, comprising administering an IL-21 polynucleotide or fragment thereof, in an amount effective to treat the immune-related disease.

10. A method of treating an immune-related disease in a mammal, comprising administering an expression vector containing an IL-21 polynucleotide, in an amount effective to treat the immune-related disease.

11. The method according to claim 10, wherein the expression vector is pORF.

12. A method of preventing a cancer in a mammal, comprising administering an IL-21 polypeptide, homologue, or fragment thereof, in an amount effective to prevent the cancer.

13. A method of preventing a cancer in a mammal, comprising administering an IL-21 polynucleotide or fragment thereof, in an amount effective to prevent the cancer.

14. A method of preventing a cancer in a mammal, comprising administering an expression vector containing an IL-21 polynucleotide, in an amount effective to prevent the cancer.

15. The method according to claim 14, wherein the expression vector is pORF.

16. The method according to claim 12, 13, 14, or 15, wherein the cancer is a melanoma.

17. The method according to claim 12, 13, 14, or 15, wherein the cancer is a sarcoma.

18. The method according to claim 12, 13, 14, or 15, wherein the cancer is a colon cancer.

19. A method of preventing a cancer in a mammal, comprising administering a vaccine formulation comprising an IL-21 polypeptide or IL-21 gene in an amount effective to induce an immunological response in the mammal to the IL-21 polypeptide, thereby prevent the cancer.

20. The method according to claim 19, wherein the IL-21 gene encoding an IL-21 polypeptide is constructed in an expression vector.

21. The method according to claim 17, wherein the expression vector is pORF.

22. The method according to claim 19, 20, or 21, wherein the cancer is a melanoma.

23. The method according to claim 19, 20, or 21, wherein the cancer is a sarcoma.

24. The method according to claim 19, 20, or 21, wherein the cancer is a colon cancer.

25. A pharmaceutical composition comprising an IL-21 polypeptide, or fragment thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

26. A pharmaceutical composition comprising an IL-21 nucleic acid, or fragment thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

27. The pharmaceutical composition according to claim 26, wherein the IL-21 nucleic acid is constructed in an expression vector.

28. The pharmaceutical composition according to claim 27, wherein the expression vector is pORF.

ABSTRACT

The present invention relates to a method of treating and/or preventing cancer in a subject, preferably mammalian, more preferably human, by administering in an effective amount IL-21 polypeptide, polynucleotide, vector comprising an IL-21 nucleic acid sequence encoding an IL-21 polypeptide, variants, homologues, and fragments thereof, thereby acting as an anti-cancer agent by reducing, ameliorating, and/or eliminating the cancer. A further embodiment encompasses a method of treating and/or preventing cancer in a subject by co-administering the IL-21 polypeptide, polynucleotide, IL-21 vector, variant, homologue, and fragments thereof, with an immunotherapeutic and/or chemotherapeutic agent for the treatment and/or prevention of cancer in a subject. In addition, the IL-21 anti-cancer agent may be beneficially administered in a low dosage with less toxicity than other cytokines used for treating or preventing cancers.

FIGURE 1

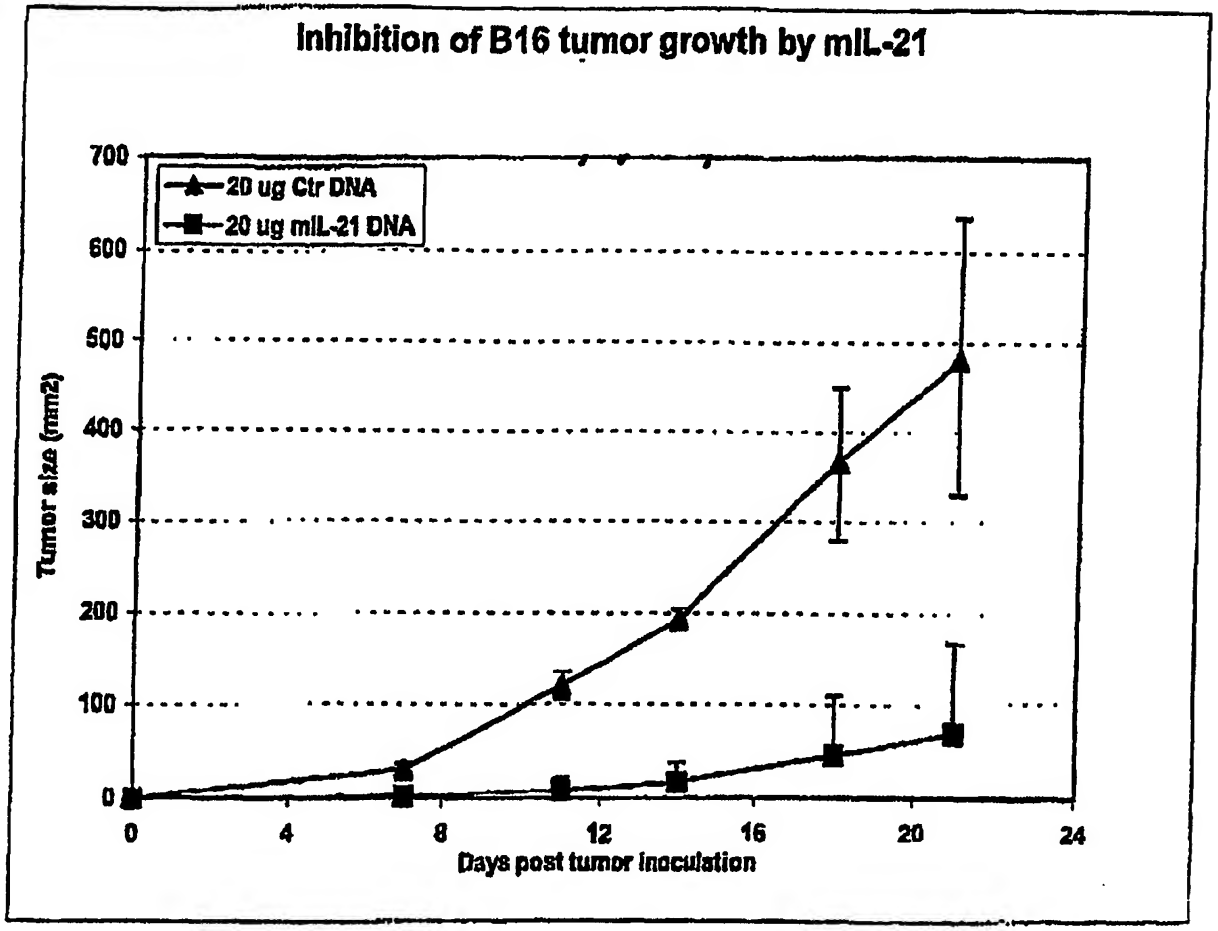

Inhibition of B16 tumor growth by mIL-21
20 ug Ctr DNA
20 ug mIL-21 DNA
Tumor size (mm2)
700
600
500
400
300
200
100
0
0
4
8
12
16
20
24
Days post tumor inoculation

FIGURE 2

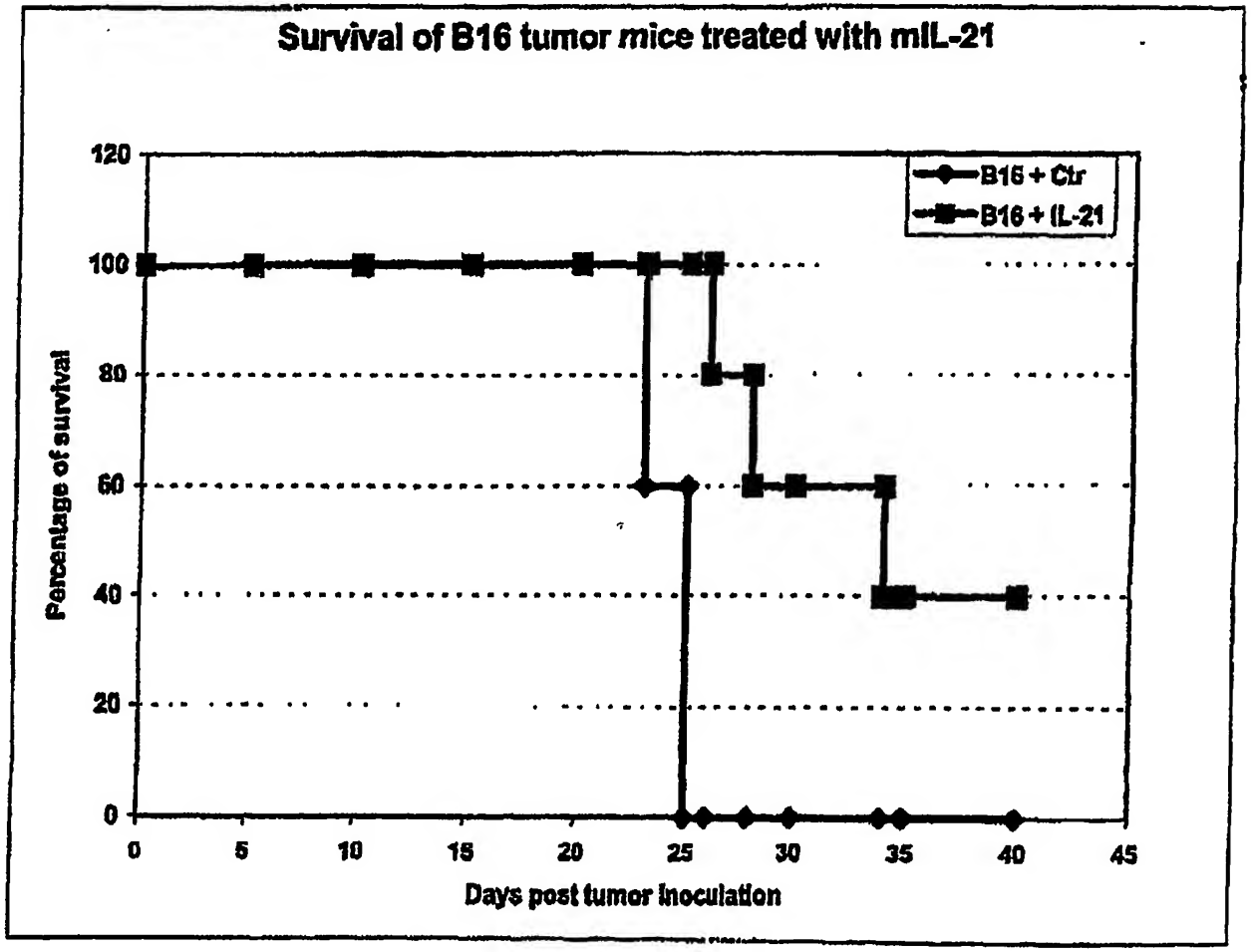

Survival of B16 tumor mice treated with mIL-21
B16 + Ctr
B16 + IL-21
Percentage of survival
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Days post tumor inoculation

FIGURE 3

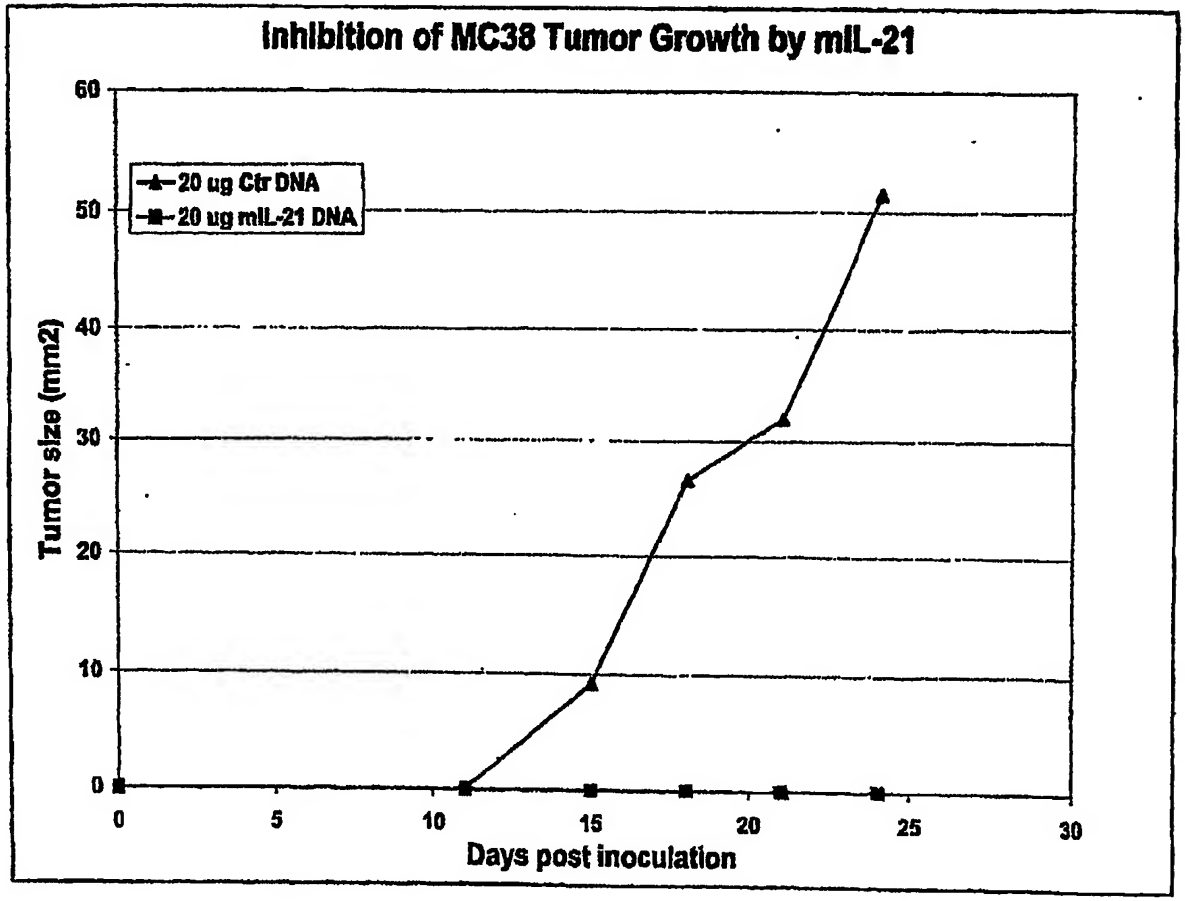

Inhibition of MC38 Tumor Growth by mIL-21
20 ug Ctr DNA
20 ug mIL-21 DNA
Tumor size (mm2)
Days post inoculation
0
10
20
30
40
50
60
0
5
10
15
20
25
30

FIGURE 4

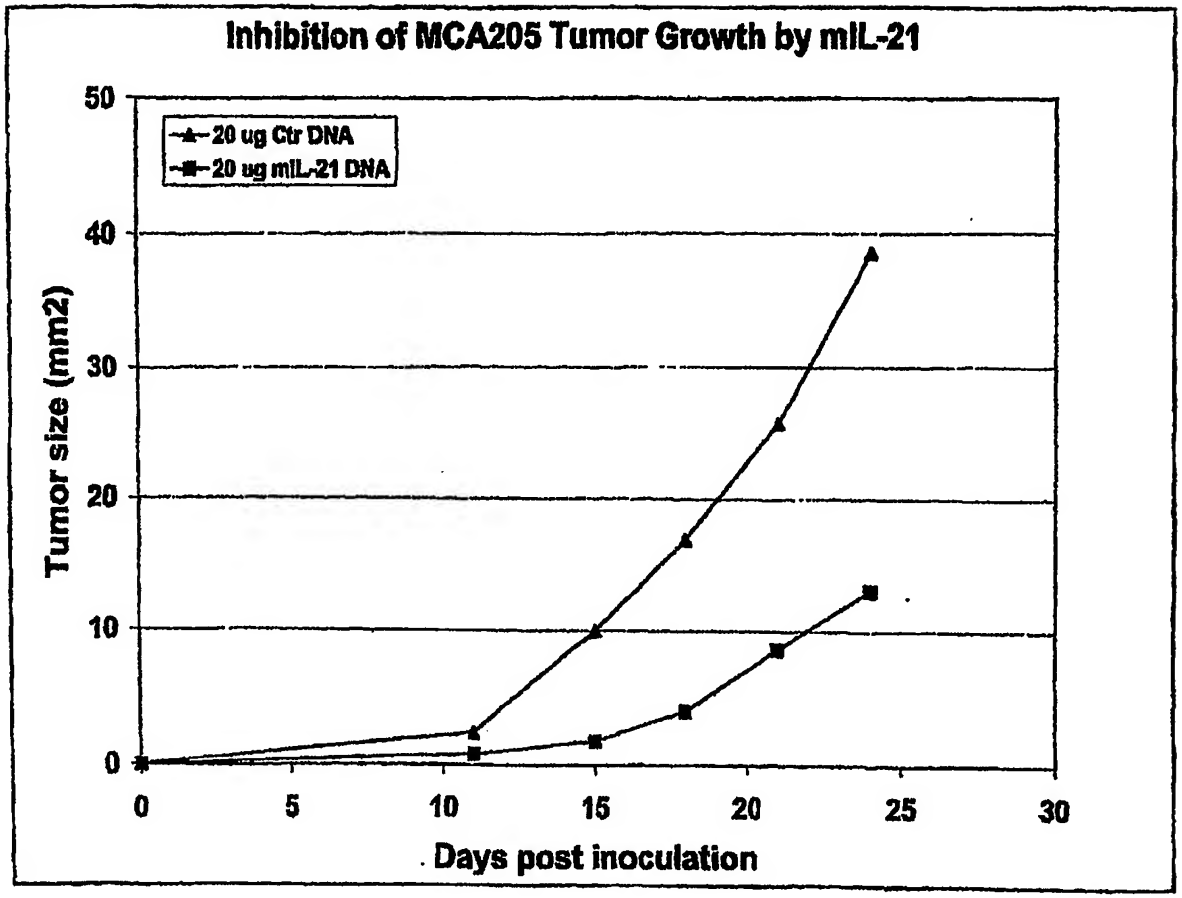

Inhibition of MCA205 Tumor Growth by mIL-21
20 ug Ctr DNA
20 ug mIL-21 DNA
Tumor size (mm2)
50
40
30
20
10
0
0
5
10
15
20
25
30
Days post inoculation

FIGURE 5

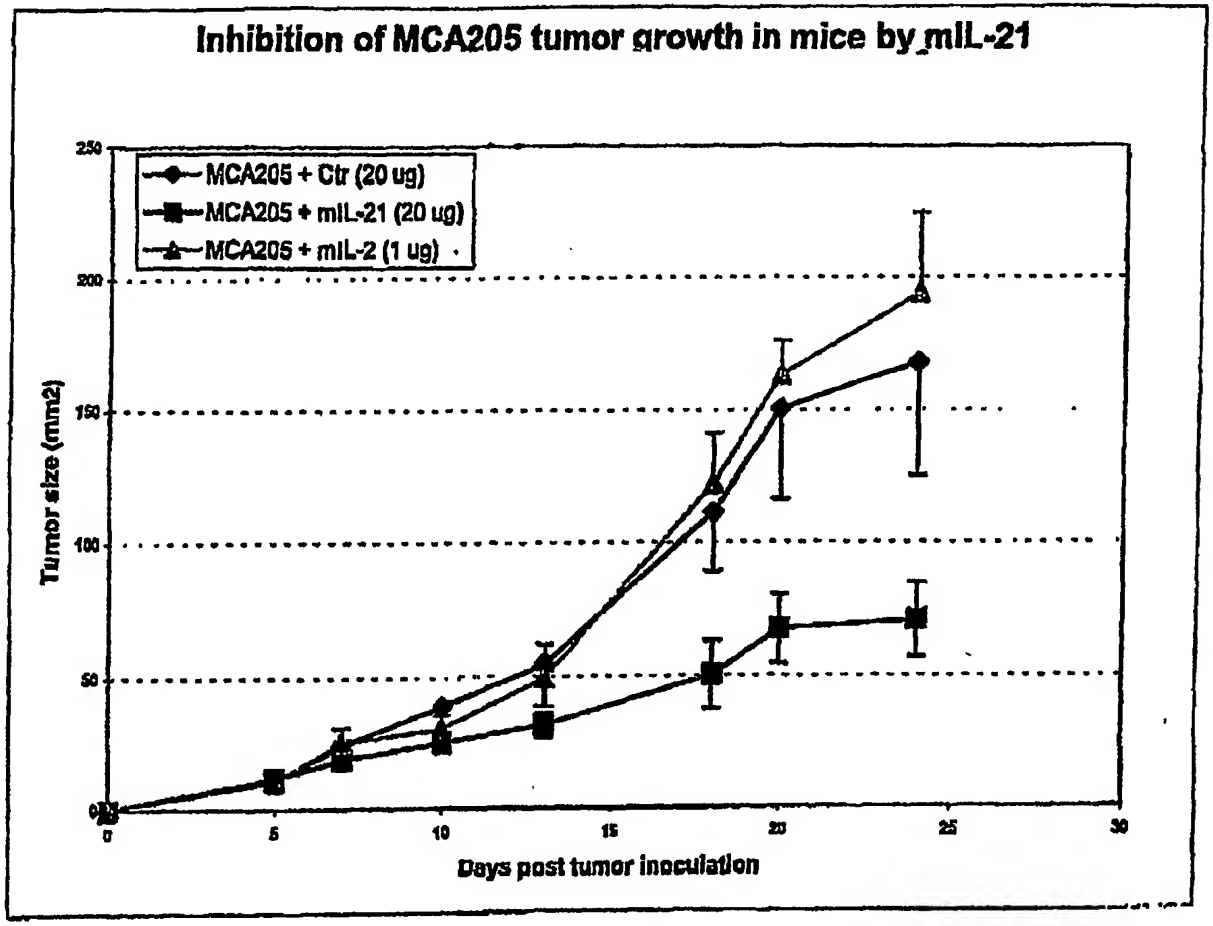
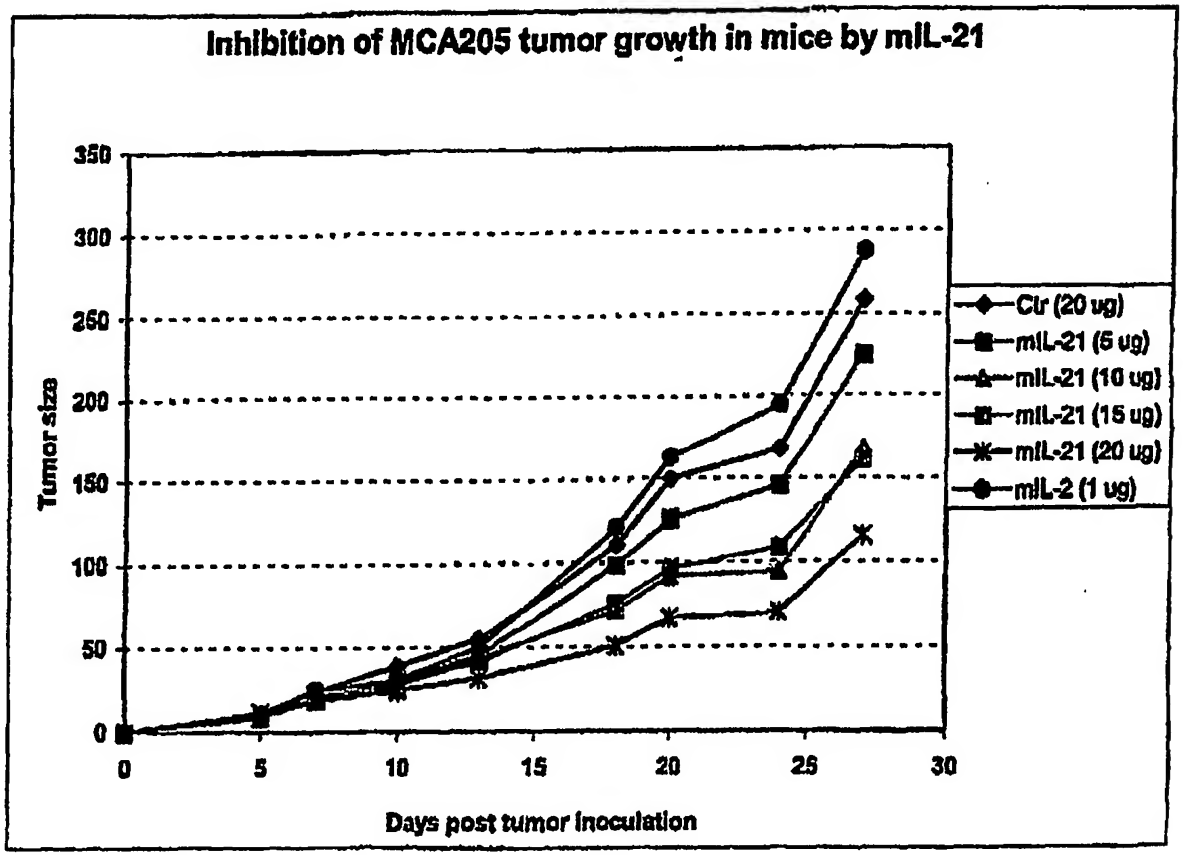

Inhibition of MCA205 tumor growth in mice by mIL-21
MCA205 + Ctr (20 ug)
MCA205 + mIL-21 (20 ug)
MCA205 + mIL-2 (1 ug)
Tumor size (mm2)
250
200
150
100
50
0
0
5
10
15
20
25
30
Days post tumor inoculation

FIGURE 6


Inhibition of MCA205 tumor growth in mice by mIL-21
Tumor size
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Days post tumor inoculation
Ctr (20 ug)
mIL-21 (5 ug)
mIL-21 (10 ug)
mIL-21 (15 ug)
mIL-21 (20 ug)
mIL-2 (1 ug)